



King's Research Portal

DOI:

[10.1097/WCO.0000000000000367](https://doi.org/10.1097/WCO.0000000000000367)

Document Version

Publisher's PDF, also known as Version of record

[Link to publication record in King's Research Portal](#)

Citation for published version (APA):

White, M. A., & Sreedharan, J. (2016). Amyotrophic lateral sclerosis: recent genetic highlights. *Current Opinion in Neurology*, 29(5), 557-64. DOI: 10.1097/WCO.0000000000000367

Citing this paper

Please note that where the full-text provided on King's Research Portal is the Author Accepted Manuscript or Post-Print version this may differ from the final Published version. If citing, it is advised that you check and use the publisher's definitive version for pagination, volume/issue, and date of publication details. And where the final published version is provided on the Research Portal, if citing you are again advised to check the publisher's website for any subsequent corrections.

General rights

Copyright and moral rights for the publications made accessible in the Research Portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognize and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the Research Portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the Research Portal

Take down policy

If you believe that this document breaches copyright please contact librarypure@kcl.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.



Amyotrophic lateral sclerosis: recent genetic highlights

Matthew A. White and Jemeen Sreedharan

Purpose of review

Amyotrophic lateral sclerosis (ALS), like other neurodegenerative diseases, remains incurable, but gene mutations linked to ALS are providing clues as to how to target therapies. It is important for researchers to keep abreast of the rapid influx of new data in ALS, and we aim to summarize the major genetic advances made in the field over the past 2 years.

Recent findings

Significant variation in seven genes has recently been found in ALS: *TBK1*, *CCNF*, *GLE1*, *MATR3*, *TUBA4A*, *CHCHD10* and *NEK1*. These have mostly been identified through large exome screening studies, though traditional linkage approaches and candidate gene screening remain important. We briefly update *C9orf72* research, noting in particular the development of reagents to better understand the normal role of *C9orf72* protein.

Summary

Striking advances in our understanding of the genetic heterogeneity of ALS continue to be made, year on year. These implicate proteostasis, RNA export, nuclear transport, the cytoskeleton, mitochondrial function, the cell cycle and DNA repair. Functional studies to integrate these hits are needed. By building a web of knowledge with interlinked genes and mechanisms, it is hoped we can better understand ALS and work toward effective therapies.

Keywords

amyotrophic lateral sclerosis, *C9orf72*, genetics, RNA processing, TDP-43

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is the most common motor neuron disease of adults and is invariably fatal, often within 5 years of disease onset. Importantly, ALS is on a clinical, genetic and pathological spectrum with frontotemporal dementia (FTD). Riluzole, a glutamate modulator and the only disease-modifying agent widely used for ALS, has only a modest effect on survival. Knockdown gene therapies hold promise for those individuals with ALS-causing gene mutations in *SOD1* and *C9orf72*, and clinical trials are underway. However, the majority of ALS is not caused by mutations in these or other identified genes. These apparently sporadic amyotrophic lateral sclerosis (sALS) cases account for around 90% of all ALS, whereas familial amyotrophic lateral sclerosis (fALS), usually dominantly inherited, accounts for the remainder.

The distinction between sALS and fALS, though practical, is increasingly recognized to be artificial, but what is important is that sALS and fALS are often indistinguishable. This suggests that ALS-linked

genes in rare fALS cases can inform our understanding of sALS. With this in mind, the dizzying pace of gene discovery in ALS in the past 10 years is to be welcomed, and advances in exome and genome sequencing strategies have been invaluable in this task. Identifying genetic risk factors and modifiers that influence ALS risk in sALS is also adding to our understanding of the mechanisms underlying ALS as a whole.

In this review, we will highlight the latest developments in the genetics of ALS focussing on genes identified in the past 2 years. Refer to Table 1 for a

The Babraham Institute, Cambridge, UK

Correspondence to Jemeen Sreedharan, Babraham Institute, Cambridge CB22 3AT, UK. Tel: +44 0 1223 496399; e-mail: jemeen.sreedharan@babraham.ac.uk

Curr Opin Neurol 2016, 29:557–564

DOI:10.1097/WCO.0000000000000367

This is an open access article distributed under the Creative Commons Attribution License 4.0, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

KEY POINTS

- The exponential rise in genetic discovery in ALS continues unabated and will continue to do so for some time.
- New genes implicate proteostasis, RNA export, nuclear transport, the cytoskeleton, mitochondrial function, the cell cycle and DNA repair.
- Novel and sophisticated approaches are needed to integrate and model this new knowledge to identify therapeutic targets.

summary of the genes primarily discussed in this article. We will then highlight recent advances in our understanding of *C9orf72*, still the hottest topic in the field. Readers are encouraged to refer to the Amyotrophic Lateral Sclerosis Online Genetics Database website (<http://alsod.iop.kcl.ac.uk/>) for a comprehensive list of other *ALS* genes or Table 2 for a summary.

NEW ALS GENES

TBK1

Perhaps the most significant recent genetic discovery in ALS since *C9orf72* has been the identification of mutations in the *TBK1* gene (encoding TANK

binding kinase 1). One study examined 2869 ALS patients and 6405 controls and found nearly 50 variants throughout *TBK1* in ALS patients, including splice-site, loss-of-function (LoF) and missense mutations [1[¶]]. Another study used whole exome sequencing and linkage analysis to interrogate 252 fALS cases that were negative for *SOD1* and *C9orf72* mutation [2^{¶¶}]. In this study, eight predicted LoF mutations in *TBK1* were found in 13 families (a mixture of truncating and splice-site mutations). The mean age of onset of fALS was 60 years of age, 15% with bulbar onset and with 50% of cases demonstrating cognitive impairment, often frank FTD. LoF mutations were not a contributor to sALS, but nine missense variants were found in both fALS and sALS.

The *TBK1* LoF mutations would be predicted to truncate TBK1, but seven of the eight variants identified by Freischmidt *et al.* produced little or no detectable transcripts or protein products, suggesting that haploinsufficiency of TBK1 is responsible for neurodegeneration. The most 3' truncation mutation [c.2138+2T>C (p.690–713del)] generated a protein product lacking 22 amino acids from the C-terminal coiled-coil domain 2, which appeared to abolish its ability to bind optineurin (OPTN) *in vitro*. Several of the missense mutations also abrogated the ability of TBK1 to bind OPTN and/or phosphorylate IRF3. It remains unclear how loss of TBK1 function causes neurodegeneration, but aberrant cellular clearance is a possibility given that both OPTN and p62, another ALS-linked protein that TBK1 interacts with, have roles in autophagy [3,4].

TBK1 interacts with OPTN, which binds to ubiquitin chains on mitochondria. This recruits TBK1 to mitochondria and promotes its kinase activation. TBK1 can then phosphorylate the UBAN domain of OPTN, expanding its ability to bind ubiquitin chains. This positive feedback mechanism promotes recruitment and retention of TBK1/OPTN to damaged mitochondria [5]. Further evidence for a role of TBK1/OPTN in the removal of damaged mitochondria involves the PINK1-PARKIN pathway and mitophagy through the polyubiquitination of defective mitochondria. TBK1 physically associates with autophagy adapters OPTN, NDP52 and p62, strengthening the link between ALS and selective autophagy [6], and TBK1 is able to phosphorylate p62 at S403 in the early period of mitochondrial depolarization [7].

Further *TBK1* variants have recently been found in European, Taiwanese and Chinese patients with ALS and/or FTD [8–11]. Strikingly, up to 10.8% of cases of patients presenting with ALS–FTD in a French patient cohort were found to carry LoF mutations in *TBK1*, suggesting that patients

Table 1. Article overview

We aim to summarize the major genetic advances in the field of ALS over the past 2 years:

Significant variation in seven genes have recently been found in ALS:

TBK1 (TANK binding kinase 1) mutations implicate abnormal cellular clearance in ALS and TBK1-ALS could be seen as yet another 'TDP-43 proteinopathy'

CCNF encodes cyclin F and implicates abnormal protein ubiquitination and possible aberrant cell cycle re-entry as mechanisms of neuronal loss in ALS

GLE1 encodes two isoforms involved in multiple cellular functions including nuclear export and stress granule function

MATR3 mutations have been implicated in ALS. Matrin 3 is a 125-kDa nuclear matrix protein that binds DNA and RNA

TUBA4A encoding the Tubulin, Alpha 4A protein further implicates cytoskeletal structural dynamics in ALS pathogenesis

CHCHD10 mutations further implicate mitochondrial dysfunction in ALS

NEK1 mutations are of interest as NEK1 interacts with two known ALS proteins, Alsin and VAPB

Advances in our understanding of *C9orf72* are discussed covering possible mechanisms of disease, development of tools to further study the gene and oligogenicity in ALS

ALS, amyotrophic lateral sclerosis.

Table 2. Genes implicated in amyotrophic lateral sclerosis

ALS type	Gene ID	Description	Location
ALS 1	SOD1	Superoxide dismutase 1, soluble	21q22.11
ALS 2	ALS2	ALS2, alsin Rho guanine nucleotide exchange factor	2q33.2
ALS 3	ALS3	Amyotrophic lateral sclerosis 3 (autosomal dominant)	18q21
ALS 4	SETX	Senataxin	9q34.13
ALS 5	SPG11	Spastic paraplegia 11 (autosomal recessive)	15q14
ALS 6	FUS	FUS RNA binding protei	16p11.2
ALS 7	ALS7	Amyotrophic lateral sclerosis 7	20p13
ALS 8	VAPB	VAMP (vesicle-associated membrane protein) associated protein B and C	20q13.33
ALS 9	ANG	Angiogenin	14q11.1
ALS 10	TARDBP	TAR DNA binding protein	1p36.22
ALS 11	FIG4	FIG4 phosphoinositide 5-phosphatase	6q21
ALS 12	OPTN	Optineurin	10p13
ALS 13	ATXN2	Ataxin 2	12q24.1
ALS 14	VCP	Valosin containing protein	9p13.3
ALS 15	UBQLN2	Ubiquilin 2	Xp11.21
ALS 16	SIGMAR1	Sigma nonopioid intracellular receptor 1	9p13.3
ALS 17	CHMP2B	Charged multivesicular body protein 2B	3p11.2
ALS 18	PFN1	Profilin 1	17p13.3
ALS 19	ERBB4	erb-b2 receptor tyrosine kinase 4	2q33.3-q34
ALS 20	HNRNPA1	Heterogeneous nuclear ribonucleoprotein A1	12q13.1
ALS 21	MATR3	Matrin 3	5q31.2
ALS-FTD1	C9orf72	Chromosome 9 open reading frame 72	9p21.2
ALS-FTD2	CHCHD10	Coiled-coil-helix-coiled-coil-helix domain containing 10	22q11.23
ALS	UNC13A	unc-13 homolog A (<i>C. elegans</i>)	19p13.11
ALS	DAO	D-amino-acid oxidase	12q24
ALS	DCTN1	Dynactin subunit 1	2p13
ALS	NEFH	Neurofilament, heavy polypeptide	22q12.2
ALS	PRPH	Peripherin	12q13.12
ALS	SQSTM1	Sequestosome 1	5q35
ALS	TAF15	TATA-box binding protein associated factor 15	17q12
ALS	SPAST	Spastin	2p24-p21
ALS	ELP3	Elongator acetyltransferase complex subunit 3	8p21.1
ALS	LMNB1	Lamin B1	5q23.2
	GLE1	GLE1 RNA export mediator	9q34.11
	TBK1	TANK binding kinase 1	12q14.1
	TUBA4A	Tubulin alpha 4a	2q35
	CCNF	Cyclin F	16p13.3
	NEK1	NIMA related kinase 1	4q33

ALS, amyotrophic lateral sclerosis; FTD, frontotemporal dementia.

presenting with FTD and ALS who do not have *C9orf72* mutations should be screened for *TBK1* mutations [12]. In another study of 104 pathologically confirmed FTLT-TDP patients who were negative for *C9orf72* and *GRN* mutations, 4.8% of patients had mutations in *TBK1*, *OPTN* or both [13]. These mutations were associated with reduced expression of *TBK1* and/or *OPTN* mRNA and protein in cerebellar tissue. This study implicates the OPTN/

TBK1 pathway in FTD with TDP-43 inclusions [13]. Pathological analysis of brain and spinal cord from ALS patients with *TBK1* mutations also identified TDP-43 positive inclusions [11,14]. Thus, *TBK1*-ALS is yet another 'TDP-43 proteinopathy'.

CCNF

Linkage analysis followed by whole-exome sequencing of a large ALS-FTD kindred of British ancestry

identified a missense mutation in the *CCNF* gene [15^{***}]. Further novel *CCNF* variants were identified through screening additional fALS (~600), sALS (~500) and FTD (nearly 200) cases from Europe, North America, Japan and Australia. Five novel missense mutations were found in familial ALS and/or FTD, and 19 mutations (missense, nonsense and frameshift) were seen in sporadic ALS and/or FTD. Most mutations affected highly conserved residues, and none were seen in controls. No clear genotype–phenotype pattern was evident. An additional replication study of 611 sALS cases and 1424 controls demonstrated an enrichment of mutations in ALS. In summary, mutations in *CCNF* occur throughout the world and account for 0.6–3.3% of fALS cohorts.

CCNF encodes cyclin F, a ubiquitously expressed protein, which is known to function in two major biological processes. First, cyclin F helps to form an E3 ubiquitin-protein ligase complex and transfer activated ubiquitin to target proteins for degradation. Overexpression of ALS mutant cyclin F isoforms appeared to result in an increase in ubiquitinated proteins and, notably, an increase in TDP-43 ubiquitination [15^{***}]. Whether the underlying mechanism is due to a toxic gain of function and whether cyclin F-linked ALS is a ‘TDP-43 proteinopathy’ *in vivo* are important questions requiring further investigation.

Cyclin F also has a role in coordinating the cell cycle. Cyclin F levels rise through interphase, peaking in G2 and then dropping at mitosis [16]. Cyclin F is not a highly abundant protein and appears unstable. Unlike other cyclins, its degradation is independent of the ubiquitin proteasome system but requires the C-terminal PEST region of the protein [17]. Cyclin F is a substrate binding subunit of SCF (Skp1-Cul1-F-box protein) ubiquitin ligase complexes and interacts with the ribonucleotide reductase family member 2. Ribonucleotide reductase plays a critical role in the conversion of ribonucleotides to deoxyribonucleotides necessary for replicative and repair DNA synthesis.

Attempts by postmitotic neurons to re-enter the cell cycle can lead to apoptosis and neuronal loss characterized by increased expression of cyclins. In a neurotrophin withdrawal model of primary motor neuron apoptotic cell death, nuclear condensation and DNA fragmentation can be observed along with activation of caspase-3 and caspase-9. The cyclin-dependent kinase inhibitors olomoucine, roscovitine and flavopiridol have been demonstrated to suppress the death of motoneurons. Taken together with the observation that cyclin D1 and cyclin E expression rises following removal of neurotrophic support, it can be envisaged that postmitotic neuronal loss occurs as neurons attempt to re-enter

the cell cycle inappropriately [18]. Treatment with nerve growth factor decreases cyclin F levels in PC12EY cells suggesting that cyclin F is involved in NGF-mediated cell cycle events during the differentiation of these cells [19].

GLE1

Recessive mutations in *GLE1*, encoding the RNA-processing protein Gle1, cause the lethal congenital contracture syndrome 1 and lethal arthrogryposis with anterior horn cell disease [20,21]. Candidate screening of *GLE1* in 173 fALS and 760 sALS patients recently identified novel nonsense and missense mutations in two sALS cases and a splice-site mutation in a fALS case [22^{***}]. The nonsense transcript produces no functional protein, whereas the splice-site mutation alters the C-terminus, preventing the mutant isoform from localizing at the nuclear pore where it is critical for mRNA export. These *GLE1* mutants also failed to rescue motor neuron pathology in zebrafish lacking *GLE1*. Thus, the mutations seem to cause a critical loss of function.

GLE1 encodes two isoforms, Gle1A and Gle1B. Gle1B localizes to the nuclear pore complex and shuttles between the nucleus and cytoplasm [23] and self-associates via its coiled-coil domain forming oligomers necessary for its function in mRNA export [24]. The unique C-terminal 43 amino acid region of Gle1B mediates binding to the C-terminal non-FG region of the nucleoporin CG1/NPL1 [23], whereas nuclear rim localization of Gle1B is dependent on 29 N-terminal residues that interact with Nup155 [25]. These observations are interesting given that nuclear transport has recently been implicated in motor neuron degeneration [26,27].

Gle1 also has important roles in translation termination [28,29] and initiation [30]. Interestingly, the Gle1A isoform specifically has been found to associate in stress granules, which is an area of intense research in ALS [31]. Furthermore, overexpression of Gle1A or a disease-associated isoform led to the formation of cytoplasmic protein aggregates [32]. This suggests that, rather than loss of function, cellular stress may occur through increased cytoplasmic Gle1 activity.

MATR3

Exome sequencing of a family of European ancestry with dominantly inherited ALS and dementia identified a mutation in the *MATR3* gene causing a F115C missense change [33^{***}]. Analysis of a further 108 fALS cases identified a T622A mutation in a 66-year-old Sardinian and a first cousin who presented with

rapidly progressive ALS at 64 years of age. Analysis of 96 British ALS cases identified a P154S variant in a sALS case. Further large screens of European, Canadian and Australian ALS cohorts identified three variants in *MATR3* including a V394M missense mutation and two splice-site mutations [34–36], whereas a Taiwanese study of ~200 cases found just one mutation (A72T) in a patient with bulbar-onset ALS [37]. Overall, *MATR3* mutations may account for less than 1% of ALS. Interestingly, there is clinical heterogeneity in the phenotype associated with *MATR3* mutations, with some cases having very slow progression longer than 15 years, or clinical and electromyographic findings more in keeping with distal myopathy with vocal cord paresis [38–40].

Matrin 3 is a 125-kDa nuclear matrix protein that binds DNA and RNA. It remains unclear how ALS-linked mutations might cause disease, with initial pathological and cellular studies showing little or no mislocalization of matrin 3 [33⁴¹]. There is some evidence that TDP-43 and matrin 3 may interact [42] but more work is needed to determine if *MATR3*-ALS is indeed a TDP-43 proteinopathy.

TUBA4A

Identification of *TUBA4A* (encoding the Tubulin, Alpha 4A protein) as a candidate gene associated with fALS is intriguing as it further implicates cytoskeletal structural dynamics in ALS pathogenesis [43⁴⁴]. Exome sequencing and rare variant analysis of a discovery cohort identified five nonsynonymous *TUBA4A* changes within exon 4. These changes included four missense variants – R320C/H, R215C, A383T and a nonsense mutation W407X. Four of these mutations were predicted to be deleterious, whereas three nonsynonymous changes found in 4300 controls were predicted to be benign. A replication study revealed an excess of *TUBA4A* mutations among patients and identified a T145P variant that segregated with disease. With the exception of A383T and G43V, no ALS-associated *TUBA4A* mutations were seen in 13 023 controls. Clinically, patients with *TUBA4A* mutations display spinal-onset ALS with both upper and lower motor neuron features with a low prevalence of FTD. Time will tell how common a cause of ALS *TUBA4A* mutations are.

In-vitro studies suggested that *TUBA4A* mutations confer a dominant-negative property on the protein. Mutant *TUBA4A* is inefficient at forming α -tubulin/ β -tubulin dimers and poorly incorporates into microtubules *in vitro*. Variants also inhibit the general assembly of the cellular

microtubule network and thus reduce structural stability. *TUBA4A*^{W407X} can form aggregates and therefore may sequester other tubulin binding proteins or impair cellular proteostasis.

CHCHD10

Bannwarth *et al.* [44⁴⁵] described a large kindred with slowly progressive ALS, FTD, cerebellar ataxia, mitochondrial myopathy and mitochondrial DNA deletions. Failing to identify mutations in genes normally associated with mitochondrial disease, they undertook whole exome sequencing and identified a missense mutation in *CHCHD10* (c176C>T; p.S59L), which encodes the coiled-coil helix domain-containing protein 10. This same mutation was also identified in another kindred with classical ALS–FTD, with or without Parkinsonism. *CHCHD10* is a mitochondrial protein located in the intermembrane space and enriched at cristae junctions. Both endogenous and overexpressed *CHCHD10*^{S59L} altered mitochondrial cristae ultrastructure and caused fragmentation of the mitochondrial network. Respiratory chain deficiency was also observed, suggesting that *CHCHD10* is critical for maintaining ATP production and oxygen consumption [44⁴⁵]. Mitochondria from *CHCHD10* mutant fibroblasts also showed poor genome repair after oxidative stress [45].

Subsequent studies have identified *CHCHD10* mutations in European populations in association with a variety of phenotypes, mostly ALS and FTD, but also Charcot–Marie–Tooth type 2 and spinal muscular atrophy [46–55]. In these populations, *CHCHD10* mutation accounts for less than 1% of ALS and FTD. Interestingly, studies from China suggest that although *CHCHD10* mutations are also rare in ALS, they may be a more significant cause of FTD, perhaps accounting for nearly 10% of cases in this population [56–58].

NEK1

Another minor hit from the exome sequencing study of Cirulli *et al.* [1⁴⁶] was dominant LoF mutations in *NEK1*. More recently, this gene was implicated in a separate whole exome sequencing study of 265 fALS index cases and 827 control individuals [59]. More screens will help determine how common a cause of ALS and/or FTD *NEK1* mutations are, but it is notable that initial studies suggest that *NEK1* interacts with two known ALS proteins, *Alsin* and *VAPB* [1⁴⁶].

Other studies have shown that the *C21orf2* protein can interact with *NEK1*. *C21orf2* affects cell proliferation after DNA damage. DNA repair was

shown to be less efficient in C21ORF2-depleted cells and homologous recombination was impaired. This deficit could be rescued by the overexpression of NEK1 [60]. NEK1 is a member of the NIMA (never in mitosis A) related kinase family of serine/threonine kinases involved in the early cellular response to genotoxic stress and plays an important role in preventing cell death induced by DNA damage [61,62]. Foci of damaged DNA in NEK1 null cells remain after removal of a toxic insult, and NEK1 null cells develop unstable chromosomes at a rate much higher than identically cultured wild-type cells [63]. NEK1 also plays a role in mitochondrial function regulating a pathway of mitochondrial cell death through phosphorylation of voltage-dependent anion channel 1 (VDAC1) on serine 193 [64].

LATEST DEVELOPMENTS IN C9orf72

The *C9orf72* gene remains the most lucrative area of research in ALS right now. The astounding progress in our understanding of *C9orf72* biology is due to the novelty of the gene mutation, an intronic hexanucleotide repeat expansion (HRE) and myriad questions regarding its mode of toxicity; the frequency of the mutation, which accounts for 22–61% of fALS, 6–19% of sALS and around 25% of familial FTD in European populations; the fact that *C9orf72* mutation is associated with TDP-43 pathology, the hallmark protein of ALS and half of FTD cases and the fact that *C9orf72* links both ALS and FTD, which are on a clinicopathological spectrum. The history and progress of ‘C9-ALS-FTD’ have recently been well reviewed elsewhere [65–67], but a few points are worthy of note.

- (1) Although the debate over protein (dipeptide repeats, DPRs, generated by RAN translation) and RNA toxicity (RNA foci, G-quadruplexes, sequestration of RNA-binding proteins) in C9-ALS-FTD continues, mouse knockout studies have shown that loss of *C9orf72*, while not obviously toxic to the nervous system, can cause severe immune dysregulation and lymphoma [68–70]. One study suggested a role for *C9orf72* in autophagy [71]. It has therefore been suggested that human *C9orf72* knockdown therapies should specifically target the HRE-containing allele to avoid the potential effects of complete loss of *C9orf72*.
- (2) The recent development of novel antibodies directed against the short and long isoforms of the *C9orf72* protein is welcome [72^{***}]. These tools demonstrated that the long isoform is present in the cytoplasm *in vivo*. The short isoform localizes to the nuclear membrane, but in

ALS patients it seems to relocate to the plasma membrane, and in these cells TDP-43 mislocalizes as well [72^{***}]. These results are intriguing given recent interest in nucleocytoplasmic transport disruption in models of *C9orf72* mutation (reviewed in [27]). More experimental tools are needed to elucidate the normal functions of *C9orf72* protein, whose biology remains poorly understood.

- (3) Novel transgenic mouse models have recently been developed, which variably recapitulate features of ALS [73–76,77^{***}]. One BAC-transgenic model in particular develops physical and pathological phenotypes relevant to human disease, including, importantly, TDP-43 proteinopathy [77^{***}]. Intriguingly, this model found that antisense transcripts were better correlated with disease than sense transcripts. These models may prove valuable in developing biomarkers and therapies for ALS-FTD.
- (4) Oligogenicity in ALS is a topic of growing interest and appears to be more common in fALS than sALS [78]. One particularly interesting example is the coexistence of intermediate expansions in *ATXN2* with the *C9orf72* HRE [78–80]. It has been suggested that *ATXN2* expansion may push *C9orf72* HRE carriers toward an ALS or ALS-FTD phenotype rather than one of the many other phenotypes associated with the mutation. Further genotype–phenotype studies are warranted, as determining how oligogenicity in ALS modifies phenotypes will no doubt lead to important insights into disease pathogenesis and yield credible therapeutic targets.

CONCLUDING REMARKS

Significant genetic advances continue to be made in ALS as we have summarized above. These clues implicate almost every area of cell biology, in particular proteostasis, RNA processing and the neuronal cytoskeleton. Having focussed our attention on these new genes, and advances in *C9orf72*, we have not had an opportunity to describe a number of other intriguing advances made in our understanding of established ALS genes. These include a potential role for TDP-43 in cryptic exon splicing [81], hydrogel studies of hnRNPs, DPRs and FUS, which show that ALS mutations can impair proteostasis and axonal local translation [82–85], the characterization of the first SOD1 ENU mutant knock-in mouse that develops a motor phenotype [86] and a large Turkish study identifying a number of intriguing new rare variants in ALS cases [87]. Thanks to project MinE (www.projectmine.com)

and other gene-hunting projects, we are set to identify yet more ALS-causing genes. These genetic discoveries clearly indicate the complexity of the mammalian motor system and how it can be made to suffer in so many different ways. However daunting this complexity may seem, our growing knowledge will empower us to accelerate progress toward finding ways to slow and potentially halt the neurodegeneration that occurs in ALS.

Acknowledgements

None.

Financial support and sponsorship

The current work was supported by the Medical Research Council and the Motor Neuron Disease Association (Lady Edith Wolfson Fellowship awarded to J.S.).

Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as

- of special interest
- of outstanding interest

1. Cirulli ET, Lasseigne BN, Petrovski S, *et al.* Exome sequencing in amyotrophic lateral sclerosis identifies risk genes and pathways. *Science* 2015; 347:1436.
- This publication utilized exome sequencing to identify novel amyotrophic lateral sclerosis (ALS) risk genes and pathways including autophagy and neuroinflammation through mutation of *TBK1* and *OPTN*. The study also suggests a possible association between mutation of *NEK1* and ALS implicating pathways including endosomal trafficking and endoplasmic reticulum function.
2. Freischmidt A, Wieland T, Richter B, *et al.* Haploinsufficiency of *TBK1* causes familial ALS and fronto-temporal dementia. *Nat Neurosci* 2015; 18:631–636. This study built on the earlier identification of mutations in *TBK1* as a cause of ALS with interesting functional data suggesting a loss-of-function mechanism and further implicating a combined role with optineurin in ALS pathogenesis.
3. Maruyama H, Morino H, Ito H, *et al.* Mutations of optineurin in amyotrophic lateral sclerosis. *Nature* 2010; 465:223–226.
4. Fecto F, Yan J, Vemula SP, *et al.* SQSTM1 mutations in familial and sporadic amyotrophic lateral sclerosis. *Arch Neurol* 2011; 68:1440–1446.
5. Richter B, Sliter DA, Herhaus L, *et al.* Phosphorylation of OPTN by *TBK1* enhances its binding to Ub chains and promotes selective autophagy of damaged mitochondria. *Proc Natl Acad Sci U S A* 2016; 113:4039.
6. Heo J-M, Ordureau A, Paulo JA, *et al.* The PINK1-PARKIN mitochondrial ubiquitylation pathway drives a program of OPTN/NDP52 recruitment and *TBK1* activation to promote mitophagy. *Molecular Cell* 2015; 60:7.
7. Matsumoto G, Shimogori T, Hattori N, Nukina N. *TBK1* controls autophagosome engulfment of polyubiquitinated mitochondria through p62/SQSTM1 phosphorylation. *Hum Mol Genet* 2015; 24:4429.
8. Tsai PC, Liu YC, Lin KP, *et al.* Mutational analysis of *TBK1* in Taiwanese patients with amyotrophic lateral sclerosis. *Neurobiol Aging* 2016; 40:191.e11–191.e16.
9. Williams KL, McCann EP, Fifita JA, *et al.* Novel *TBK1* truncating mutation in a familial amyotrophic lateral sclerosis patient of Chinese origin. *Neurobiol Aging* 2015; 36:3334.e1–3334.e5.
10. Borghero G, Pugliatti M, Marrosu F, *et al.* *TBK1* is associated with ALS and ALS-FTD in Sardinian patients. *Neurobiol Aging* 2016; 43:180.e1.
11. Gijssels I, Van Mossevelde S, van der Zee J, *et al.* Loss of *TBK1* is a frequent cause of frontotemporal dementia in a Belgian cohort. *Neurology* 2015; 85:2116–2125.
12. Le Ber I, De Septenville A, Millecamps S, *et al.* *TBK1* mutation frequencies in French frontotemporal dementia and amyotrophic lateral sclerosis cohorts. *Neurobiol Aging* 2015; 36:3116.e5–3116.e8.
13. Pottier C, Bieniek KF, Finch N, *et al.* Whole-genome sequencing reveals important role for *TBK1* and *OPTN* mutations in frontotemporal lobar degeneration without motor neuron disease. *Acta Neuropathol* 2015; 130:77–92.
14. Freischmidt A, Wieland T, Richter B, *et al.* Haploinsufficiency of *TBK1* causes familial ALS and fronto-temporal dementia. *Nat Neurosci* 2015; 18:631.
15. Williams KL, Topp S, Yang S, *et al.* *CCNF* mutations in amyotrophic lateral sclerosis and frontotemporal dementia. *Nat Commun* 2016; 7:11253. This interesting publication was the first to uncover a mutation of *CCNF* as a cause of ALS/FTD and provided data suggesting a role in protein ubiquitination including of TDP-43.
16. Bai C, Richman R, Elledge SJ. Human cyclin F. *EMBO J* 1994; 13:6087–6098.
17. Fung TK, Siu WY, Yam CH, *et al.* Cyclin F is degraded during G2-M by mechanisms fundamentally different from other cyclins. *J Biol Chem* 2002; 277:35140.
18. Appert-Collin A, Hugel B, Levy R, *et al.* Cyclin dependent kinase inhibitors prevent apoptosis of postmitotic mouse motoneurons. *Life Sci* 2006; 79:484–490.
19. Movsesyan V, Whalin M, Shibutani M, *et al.* Down-regulation of cyclin F levels during nerve growth factor-induced differentiation of PC12 cells. *Exp Cell Res* 1996; 227:203.
20. Nousiainen HO, Kestilä M, Pakkasjärvi N, *et al.* Mutations in mRNA export mediator *GLE1* result in a fetal motoneuron disease. *Nat Genet* 2008; 40:155.
21. Folkmann AW, Dawson TR, Wentz SR. Insights into mRNA export-linked molecular mechanisms of human disease through a *Gle1* structure–function analysis. *Adv Biol Regul* 2014; 54:74.
22. Kaneb HM, Folkmann AW, Belzil VV, *et al.* Deleterious mutations in the essential mRNA metabolism factor, *hGle1*, in amyotrophic lateral sclerosis. *Hum Mol Genet* 2014; 24:1363.
- Mounting evidence suggesting impaired nuclear import/export may play a role in ALS pathogenesis makes this publication into the role of the nuclear export protein *GLE1* of particular interest. The study suggests a mechanism of haploinsufficiency and builds on literature implicating defects in RNA metabolism in ALS.
23. Kendirgi F. Interaction between the shuttling mRNA export factor *Gle1* and the nucleoporin *hCG1*: a conserved mechanism in the export of *Hsp70* mRNA. *Mol Biol Cell* 2005; 16:4304.
24. Folkmann AW, Collier SE, Zhan X, *et al.* *Gle1* functions during mRNA export in an oligomeric complex that is altered in human disease. *Cell* 2013; 155:582.
25. Rayala HJ. The mRNA export factor human *Gle1* interacts with the nuclear pore complex protein *Nup155*. *Mol Cell Proteomics* 2003; 3:145.
26. Sreedharan J, Neukomm LJ, Brown RH Jr, Freeman MR. Age-dependent TDP-43-mediated motor neuron degeneration requires GSK3, hat-trick, and xmas-2. *Curr Biol* 2015; 25:2130–2136.
27. Jovicic A, Paul JW 3rd, Gitler AD. Nuclear transport dysfunction: a common theme in amyotrophic lateral sclerosis and frontotemporal dementia. *J Neurochem* 2016. [Epub ahead of print]
28. Bolger TA, Folkmann AW, Tran EJ, Wentz SR. The mRNA export factor *Gle1* and inositol hexakisphosphate regulate distinct stages of translation. *Cell* 2008; 134:624.
29. Alcazar-Roman AR, Bolger TA, Wentz SR. Control of mRNA export and translation termination by inositol hexakisphosphate requires specific interaction with *Gle1*. *J Biol Chem* 2010; 285:16683.
30. Bolger TA, Wentz SR. *Gle1* is a multifunctional DEAD-box protein regulator that modulates *Ded1* in translation initiation. *J Biol Chem* 2011; 286:39750.
31. Aditi. Folkmann AW, Wentz SR. Cytoplasmic *hGle1A* regulates stress granules by modulation of translation. *Mol Biol Cell* 2015; 26:1476.
32. Aditi. Glass L, Dawson TR, Wentz SR. An amyotrophic lateral sclerosis-linked mutation in *GLE1* alters the cellular pool of human *Gle1* functional isoforms. *Adv Biol Regul* 2015. [Epub ahead of print]
33. Johnson JO, Piro EP, Boehringer A, *et al.* Mutations in the *Matrin 3* gene cause familial amyotrophic lateral sclerosis. *Nat Neurosci* 2014; 17:664. This work proved to be of interest as it not only first implicated *MATR3* as a protein linked to ALS but also suggests an interaction with TDP-43. As a nucleic acid binding protein, the study provides yet more evidence for aberrant RNA processing in motor neuron pathology.
34. Millecamps S, De Septenville A, Teyssou E, *et al.* Genetic analysis of *matrin 3* gene in French amyotrophic lateral sclerosis patients and frontotemporal lobar degeneration with amyotrophic lateral sclerosis patients. *Neurobiol Aging* 2014; 35:2882.e13.
35. Fifita JA, Williams KL, McCann EP, *et al.* Mutation analysis of *MATR3* in Australian familial amyotrophic lateral sclerosis. *Neurobiol Aging* 2015; 36:1602.e1.
36. Leblond CS, Gan-Or Z, Spiegelman D, *et al.* Replication study of *MATR3* in familial and sporadic amyotrophic lateral sclerosis. *Neurobiol Aging* 2016; 37:209.e17.
37. Lin K-P, Tsai P-C, Liao Y-C, *et al.* Mutational analysis of *MATR3* in Taiwanese patients with amyotrophic lateral sclerosis. *Neurobiol Aging* 2015; 36:2005.e1.
38. Müller TJ, Kraya T, Stoltenberg-Didinger G, *et al.* Phenotype of *matrin-3*-related distal myopathy in 16 German patients. *Ann Neurol* 2014; 76:669–680.
39. Feit H, Silbergleit A, Schneider LB, *et al.* Vocal cord and pharyngeal weakness with autosomal dominant distal myopathy: clinical description and gene localization to 5q31. *Am J Hum Genet* 1998; 63:1732–1742.

40. Senderek J, Garvey SM, Krieger M, *et al.* Autosomal-dominant distal myopathy associated with a recurrent missense mutation in the gene encoding the nuclear matrix protein, matrin 3. *Am J Hum Genet* 2009; 84:511–518.
 41. Gallego-Iradi MC, Clare AM, Brown HH, *et al.* Subcellular localization of matrin 3 containing mutations associated with ALS and distal myopathy. *PLoS One* 2015; 10:e0142144.
 42. Ling SC, Albuquerque CP, Han JS, *et al.* ALS-associated mutations in TDP-43 increase its stability and promote TDP-43 complexes with FUS/TLN1. *Proc Natl Acad Sci U S A* 2010; 107:13318–13323.
 43. Smith BN, Ticozzi N, Fallini C, *et al.* Exome-wide rare variant analysis identifies TUBA4A mutations associated with familial ALS. *Neuron* 2014; 84:324–331.
- This work identifying *TUBA4A* mutations as a cause of ALS proved to be an interesting read as it builds on the role of cytoskeletal dynamics in neuron degeneration in addition to the use of gene-based rare variant analysis as a technique to identify novel disease genes.
44. Bannwarth S, Ait-El-Mkadem S, Chausseuot A, *et al.* A mitochondrial origin for frontotemporal dementia and amyotrophic lateral sclerosis through CHCHD10 involvement. *Brain* 2014; 137:2329–2345.
- The initial study implicating mitochondrial dysfunction in both mitochondrial myopathy and ALS.
45. Genin EC, Plutino M, Bannwarth S, *et al.* CHCHD10 mutations promote loss of mitochondrial cristae junctions with impaired mitochondrial genome maintenance and inhibition of apoptosis. *EMBO Mol Med* 2016; 8:58–72.
 46. Chausseuot A, Le Ber I, Ait-El-Mkadem S, *et al.* French Research Network on FTD, *et al.* Screening of CHCHD10 in a French cohort confirms the involvement of this gene in frontotemporal dementia with amyotrophic lateral sclerosis patients. *Neurobiol Aging* 2014; 35:2884.e1–2884.e4.
 47. Chio A, Mora G, Sabatelli M, *et al.* CHCHD10 mutations in an Italian cohort of familial and sporadic amyotrophic lateral sclerosis patients. *Neurobiol Aging* 2015; 36:1767.e3–1767.e6.
 48. Pasanen P, Myllykangas L, Poyhonen M, *et al.* Intrafamilial clinical variability in individuals carrying the CHCHD10 mutation Gly66Val. *Acta Neurol Scand* 2016; 133:361–366.
 49. Johnson JO, Glynn SM, Gibbs JR, *et al.* Mutations in the CHCHD10 gene are a common cause of familial amyotrophic lateral sclerosis. *Brain* 2014; 137:e311.
 50. Muller K, Andersen PM, Hubers A, *et al.* Two novel mutations in conserved codons indicate that CHCHD10 is a gene associated with motor neuron disease. *Brain* 2014; 137:e309.
 51. Dols-Icardo O, Nebot I, Gorostidi A, *et al.* Analysis of the CHCHD10 gene in patients with frontotemporal dementia and amyotrophic lateral sclerosis from Spain. *Brain* 2015; 138:e400.
 52. Wong CH, Topp S, Gkazi AS, *et al.* The CHCHD10 P34S variant is not associated with ALS in a UK cohort of familial and sporadic patients. *Neurobiol Aging* 2015; 36:2908.e17–2908.e18.
 53. Ronchi D, Riboldi G, Del Bo R, *et al.* CHCHD10 mutations in Italian patients with sporadic amyotrophic lateral sclerosis. *Brain* 2015; 138:e372.
 54. Kurzwelly D, Kruger S, Biskup S, Heneka MT. A distinct clinical phenotype in a German kindred with motor neuron disease carrying a CHCHD10 mutation. *Brain* 2015; 138:e376.
 55. Teyssou E, Chartier L, Albert M, *et al.* Genetic analysis of CHCHD10 in French familial amyotrophic lateral sclerosis patients. *Neurobiol Aging* 2016; 42:218.e1–218.e3.
 56. Jiao B, Xiao T, Hou L, *et al.* High prevalence of CHCHD10 mutation in patients with frontotemporal dementia from China. *Brain* 2016; 139:e21.
 57. Zhou Q, Chen Y, Wei Q, *et al.* Mutation screening of the CHCHD10 gene in Chinese patients with amyotrophic lateral sclerosis. *Mol Neurobiol* 2016. [Epub ahead of print]
 58. Li XL, Shu S, Li XG, *et al.* CHCHD10 is not a frequent causative gene in Chinese ALS patients. *Amyotroph Lateral Scler Frontotemporal Degener* 2016; 17:458–460.
 59. Brenner D, Müller K, Wieland T, *et al.* NEK1 mutations in familial amyotrophic lateral sclerosis. *Brain* 2016; 139:e28.
 60. Fang X, Lin H, Wang X, *et al.* The NEK1 interactor, C21ORF2, is required for efficient DNA damage repair. *Acta Biochim Biophys Sin* 2015; 47:834.
 61. Pelegrini AL, Moura DJ, Brenner BL, *et al.* Nek1 silencing slows down DNA repair and blocks DNA damage-induced cell cycle arrest. *Mutagenesis* 2010; 25:447.
 62. Feige E, Shalom O, Tsuril S, *et al.* Nek1 shares structural and functional similarities with NIMA kinase. *Biochim Biophys Acta (BBA) – Mol Cell Res* 2006; 1763:272.
 63. Chen Y, Chen C-F, Riley DJ, Chen P-L. Nek1 kinase functions in DNA damage response and checkpoint control through a pathway independent of ATM and ATR. *Cell Cycle* 2011; 10:655.
 64. Chen Y, Craigen WJ, Riley DJ. Nek1 regulates cell death and mitochondrial membrane permeability through phosphorylation of VDAC1. *Cell Cycle* 2009; 8:257.
 65. Cooper-Knock J, Kirby J, Highley R, Shaw PJ. The spectrum of C9orf72-mediated neurodegeneration and amyotrophic lateral sclerosis. *Neurotherapeutics* 2015; 12:326–339.
 66. Todd TW, Petrucelli L. Insights into the pathogenic mechanisms of Chromosome 9 open reading frame 72 (C9orf72) repeat expansions. *J Neurochem* 2016.
 67. Haeusler AR, Donnelly CJ, Rothstein JD. The expanding biology of the C9orf72 nucleotide repeat expansion in neurodegenerative disease. *Nat Rev Neurosci* 2016; 17:383–395.
 68. Atanasiu A, Decman V, White D, *et al.* C9orf72 ablation causes immune dysregulation characterized by leukocyte expansion, autoantibody production, and glomerulonephropathy in mice. *Sci Rep* 2016; 6:23204.
 69. O'Rourke JG, Bogdanik L, Yanez A, *et al.* C9orf72 is required for proper macrophage and microglial function in mice. *Science* 2016; 351:1324–1329.
 70. Sudria-Lopez E, Koppers M, de Wit M, *et al.* Full ablation of C9orf72 in mice causes immune system-related pathology and neoplastic events but no motor neuron defects. *Acta Neuropathol* 2016; 132:145–147.
 71. Sullivan PM, Zhou X, Robins AM, *et al.* The ALS/FTLD associated protein C9orf72 associates with SMCR8 and WDR41 to regulate the autophagy-lysosome pathway. *Acta Neuropathol Commun* 2016; 4:51.
 72. Xiao S, MacNair L, McGoldrick P, *et al.* Isoform-specific antibodies reveal distinct subcellular localizations of C9orf72 in amyotrophic lateral sclerosis. *Ann Neurol* 2015; 78:568–583.
- This group has developed novel antibodies that have helped characterize the normal biology of C9orf72, which has remained a black hole up to now.
73. Peters OM, Cabrera GT, Tran H, *et al.* Human C9ORF72 hexanucleotide expansion reproduces RNA foci and dipeptide repeat proteins but not neurodegeneration in BAC transgenic mice. *Neuron* 2015; 88:902–909.
 74. O'Rourke JG, Bogdanik L, Muhammad AK, *et al.* C9orf72 BAC transgenic mice display typical pathologic features of ALS/FTD. *Neuron* 2015; 88:892–901.
 75. Chew J, Gendron TF, Prudencio M, *et al.* Neurodegeneration. C9ORF72 repeat expansions in mice cause TDP-43 pathology, neuronal loss, and behavioral deficits. *Science* 2015; 348:1151–1154.
 76. Jiang J, Zhu Q, Gendron TF, *et al.* Gain of toxicity from ALS/FTD-linked repeat expansions in C9ORF72 is alleviated by antisense oligonucleotides targeting GGGGCC-containing RNAs. *Neuron* 2016; 90:535–550.
 77. Liu Y, Pattamatta A, Zu T, *et al.* C9orf72 BAC mouse model with motor deficits and neurodegenerative features of ALS/FTD. *Neuron* 2016; 90:521–534.
- This mouse model is a particularly good mimic of the human condition as not only does it have a physical phenotype but it also has C9orf72 related pathology and TDP-43 pathology.
78. Dekker AM, Seelen M, van Doormaal PT, *et al.* Large-scale screening in sporadic amyotrophic lateral sclerosis identifies genetic modifiers in C9orf72 repeat carriers. *Neurobiol Aging* 2016; 39:220.e9–220.e15.
 79. van Blitterswijk M, Mullen B, Heckman MG, *et al.* Ataxin-2 as potential disease modifier in C9ORF72 expansion carriers. *Neurobiol Aging* 2014; 35:2421.e13–2421.e17.
 80. Lattante S, Millicamps S, Stevanin G, *et al.* Contribution of ATXN2 intermediary polyQ expansions in a spectrum of neurodegenerative disorders. *Neurology* 2014; 83:990–995.
 81. Ling JP, Pletnikova O, Troncoso JC, Wong PC. TDP-43 repression of nonconserved cryptic exons is compromised in ALS–FTD. *Science* 2015; 349:650–655.
 82. Kwon I, Xiang S, Kato M, *et al.* Poly-dipeptides encoded by the C9orf72 repeats bind nucleoli, impede RNA biogenesis, and kill cells. *Science* 2014; 345:1139–1145.
 83. Xiang S, Kato M, Wu LC, *et al.* The LC domain of hnRNP A2 adopts similar conformations in hydrogel polymers, liquid-like droplets, and nuclei. *Cell* 2015; 163:829–839.
 84. Murakami T, Qamar S, Lin JQ, *et al.* ALS/FTD mutation-induced phase transition of FUS liquid droplets and reversible hydrogels into irreversible hydrogels impairs RNP granule function. *Neuron* 2015; 88:678–690.
 85. Molliex A, Temirov J, Lee J, *et al.* Phase separation by low complexity domains promotes stress granule assembly and drives pathological fibrillization. *Cell* 2015; 163:123–133.
 86. Joyce PI, McGoldrick P, Saccon RA, *et al.* A novel SOD1-ALS mutation separates central and peripheral effects of mutant SOD1 toxicity. *Hum Mol Genet* 2015; 24:1883–1897.
 87. Ozoguz A, Uyan O, Birdal G, *et al.* The distinct genetic pattern of ALS in Turkey and novel mutations. *Neurobiol Aging* 2015; 36:1764.e9–1764.e18.